81

VERIFIED TRANSLATION OF PRIORITY DOCUMENT (37 CFR 1.55(A))

I, the below-named translator, hereby declare that:

My name and post office address are as stated below.

That I am knowledgeable in the English language, and in the German language of the patent application from which priority is claimed for this application;

The priority document is attached.

I hereby state that the attached translation of the priority document that I have prepared is accurate.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

The priority document attached is further identified as:

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"Laser Scanning Microscope with Acoustic Optical Tunable Filter"

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Laser Scanning Microscope
with Acoustic Optical Tunable Filter

Description

It is a matter of current knowledge, where a laser scanning microscope laser is concerned, to introduce different wavelengths, in common, into a light conducting fiber through beam-splitters and through an Acoustic Optical Tunable Filter (hereafter, "AOTF"), which has a diffraction grating, based on index of refraction variations, the grating constant of which is variable in accord with demands of its high frequency control.

The first order wavelength diffractions by AOTF, as well as their intensity, are created by the control of the AOTF, the said intensity by the amplitude of the audio-wave and wavelength is determined by the frequency of the audio-wave.

The use of acoustic optical systems for line selection and attenuation of laser lines in a modular construction, has the disadvantage, that the transmission characteristics of the acoustic optical unit are strongly affected by operating temperature.

This is to be explained, in that with change of the temperature, one result can be a corresponding alteration in the speed of sound in crystal materials, which makes itself known indirectly in a shift from an optimal frequency, whereby a reduction of the refraction efficiency can be noted. A frequency shift of about 16 KHz / °C would be expected.

This leads to intensity losses and possible modulations of intensity in the scanned image.

If the AOTF were possibly adjusted at 21 °C, then, in a temperature change from 21 °C to 35 °C the transmitted load, without correction factors, would lose about 5 %. Even at a temperature change of only 4 °C, a load dropoff can approach 50 %.

An acceptable performance of the laser scanning microscope (hereafter LSM) is only to be attained, if the temperature variations can be limited to ± 1 °C.

This, however, is scarcely possible in practice because of the multitudinous application possibilities of the LSM.

Room temperatures of > 30 °C. moreover, are quickly achieved in many laboratories.

Thus the invention has the purpose, in spite of uncertain and non-constant setup conditions of the LSM, to guarantee the stability of the laser capacity for all required wavelengths in ultraviolet (351 nm, 364 nm) and in the visible ranges (450 nm, 650 nm).

This purpose is achieved, in accord with the invention, by the features of the independent claims.

Advantageous developments are objects of the independent claims.

In an advantageous manner, the AOTF-frequency can be controlled by the regulation of the AOTF by a driver interface dependent upon the temperature.

The capture of the temperature can be carried out, in this matter, directly proximal to the AOTF, for example, that is, directly within its housing.

In case the temperature deviation should be determined to be greater than ± 1 °C from a specified reference value, then there follows an automatic frequency reset within a predetermined window of frequencies, advantageously ± 200 KHz, at the predetermined, specified reference value (temperature) frequency. The frequency can also be adjusted, with the aid of earlier accepted and tabularly recorded temperature dependent frequency values.

The temperature deviation can also be compensated for by an intensity increase, which cancels out the efficiency loss of the AOTF.

The presented laser scanning microscope (LSM) realized on the visible light (VIS) laser module in the extreme case of the joining of one ArLaser (458 nm, 488 nm, 514 nm) or ArLaser (488 nm, 568 nm) with respectively two HeNe-lasers. The laser lines are joined on a common axis by means of dichroitic and mirror arrangements, then selected in an AOTF, whereby the first refraction of the VIS-AOTF is coupled into a single mode fiber. Using the UV laser module, a selection was made of the laser lines 351 nm and 364 nm by means of a UV-AOTF. The first refraction, likewise, was also coupled in a single mode fiber.

The invention is described and explained in greater detail with the schematic presentations of the attached Figs. 1 and 2. Shown schematically therein is, in:

Fig. 1 a beam travel path of an LSM,

Fig. 2 a perspective, profile view of an
AOTF which is provided with a heating means

In Fig. 1 is shown schematically, a microscope unit M and a scanning head S, which have a common optical interface by means of an interposed imaging Z function in accord with Fig. 2.

The scanning head S has the capability of functioning both on the photo-barrel of a vertical microscope and also on a lateral access of an inverse microscope.

Further, in Fig. 1, between a top-lighted scan and a transmitted illumination scan, by means of a pivoting, mirror 14, a non-switchable, microscope beam path is presented, with a(n):

first light source 1
illumination optical system 2
beam splitter 3
objective 4
probe 5
condenser 6
second light source 7
reception apparatus 8
first barrel line 9
second barrel lens 10 with an observation beam entry, and
ocular 11 along with a beam splitter for the coupling of the scan beam.

A laser module 13.1, 13.2 picks up the laser and is connected by means of monomode, light fibers 14.1 and 14.2 with the laser coupling unit of the scan head S.

The engagement of the light fibers 14.1 14.2 is done by means of a slidable collimation optical device 16, which will be discussed in detail below, and by beam reversal elements 17.1 and 17.2.

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By means of a partially transparent mirror 18, a monitoring beam entry is diverted in the direction of a monitor-diode 19, which, advantageously, is placed on a rotatable filter wheel (not shown) with line filter 21 as well as a neutral filter 20.

The actual scanning unit consists of a scanning objective 22, a scanner 23, a main beam splitter 24 and a common imaging optic 25 for detection channel 26.1, 26.4.

A reversal prism 27 behind the imaging optic 25 reflects the beam coming from object 5 in the direction of a dichroic beam divider 28 in the convergent beam entry of the imaging optic 25, for which, in the direction of, and perpendicular to, the optical axis, are placed variable-diameter pinholes 29, these being specific for each detection channel as well as an emission filter 30 and corresponding receiver elements 31 i.e. photomultiplier tube (PMT).

Advantageously the beam splitters 27, 28, as illustrated, can serve as a graduated wheel of multiple positions, which is switchable by means of a positioning motor.

Advantageously, there is carried out by means of an AOTF serving as a beam diverter, an engaging coupling of UV beams in the glass fibers 14.1, this being a single mode glass fiber,. In deail, if the beam should not fall on the fiber entry, then, by means of the AOTF, the beam will be diverted away from the said fiber entry, for example, diverted in the direction of a (not shown) light absorber.

The engaging coupling optic 33 of the laser beam, possesses for the said coupling a (not shown) lens system, the focal length of which is determined by both the laser beam cross-section and the required coupling necessary for the numeric aperture. Provided in the laser module 13.2 are single and multiple wavelength lasers, which, individually or commonly, by means of an AOTF are coupled into one or more fibers. Further, the engaging coupling can simultaneously be carried out on the side of the microscope after being mixed by a conditioning optical system with color unification for a multiplicity of mixed beam fibers

Also, the mixing of the emissions of varied lasers at the fiber entry is possible and, with the aid of the switchable, schematically draw, plate mirrors 39 which mirrors can be altered. .

The laser emanation from the fiber end of the fibers 14.1, 14.2 at the exit of the scan unit is collimated by the collimation optics 16 into an "unending" beam. This is done advantageously with a single lens, which has a focusing function activated by being slidingly moved along the optical axis by means of a controlling unit 37, which itself is under the regulation of a central control station. The single lens focusing function is further possible, since its separating distance to the end of the light conducting fiber in accord with the invention, is changeable.

The monitor diode 19, which also, can have a predetermined (not shown here) focusing lens, acts in conjunction with a filter wheel or filter-slider 21 which is sensitive to line or area characteristics. This said lens is under the control of regulation unit 36, for the permanent monitoring of the engaged laser radiation within the scan module, especially in order to controllingly isolate the capacity in a specific laser line, and, if necessary, to stabilize the same by the AOTF 32 by means of a control-signal to the controller 34.

The detection means of the monitor diode 19 captures laser noise and variations, by virtue of the mechanical-optical transmitting system.

From the detected momentum, laser capacity can be diverted by a fail-signal, which retroacts directly on-line to the laser or to one of the intensity modulators (ASOM, ASTF, EOM, Shutter) for the purpose of stabilization of incident laser load radiated into the scan module.

By means of the control of the filter unit 21, it is possible, that a wavelength type stabilization of the intensity and laser load control can be carried out.

By means of a connection to detection 31 (PMT) and respectively to the central control unit, by the formation of signal quotients and/or signal subtraction of the detection signal and the monitor signal of the diode 19, a noise reduction can be effected, since the corresponding sensor signal of a detection channel is presented in normalized pixel-wise manner as pixel-image-data on the signal of the monitor diode (for example, "division"), in order that, in this way the intensity fluctuations in the image may be reduced.

Directly on the AOTF is fastened a temperature sensor TE, which takes the ambient temperature which surrounds the said AOTF.

This is transmitted to the control unit 34, which contains a computer, which, with the aid of a previously input correction curve and a RS 232-driver-switching circuit, which places and optimizes the AOTF frequency in dependency of the temperature in a specified window of frequency. That is to say, the frequency displacement due to temperature or reduction is compensated. This compensation can, however, be picked up automatically with the aid of the diode 19 and communicated as part of the intensity values transmitted to the control unit, since the diode 19 is connected with the evaluation unit and the AOTF-driver adjusts the frequency with the aid of the received intensity signal of the diode 19, while the frequency, advantageously, varies some ± 100 KHZ, until a maximum signal threshold is reached.

Further, there exists an advantageous achievement therein, to provide for the AOTF with a separate heating and/or cooling means.

Especially advantageous, is that the crystal is warmed up to a range greater than 35 °C, or perhaps 40 °C and is held constant within defined limits.

The laser capacity in the first order refraction remains constant over the entire temperature range of, for example, 15 °C to 35 °C within narrow tolerances. An example of a stable temperature control, the control variances of which show no deteriorating effects for the laser-scan-microscopy, is shown in Fig. 2.

In this Fig. 2, on a housing part G, which possesses feed for voltage supply for the AOTF, is placed the TeO_2 crystal of the AOTF, wherein the penetrating laser beam is schematically indicated.

Between the housing G and the TeO_2 crystal is to be found an electrically heated or cooled plate P, the current supply ST for which can, as shown, be attached on the outer side of the housing.

The current supply ST is connection with a regulator, which in turn is connected to a temperature feeler, which can be directly placed on the TeO_2 crystal or on the current supply ST.

The control can be a part of the current supply ST but can also be carried out by the control unit 34.

The temperature feeler TF can also, as is already indicated in Fig. 1, be placed on the housing and connected with the evaluation unit 34, which captures the temperature variations and correspondingly regulates the AOTF.

CLAIMS

Claimed is:

1. A laser scanning microscope with an AOTF in the laser engagement-beam entry, whereby in the zone proximal to the AOTF or in the neighborhood thereof, or connected thereto, a temperature sensor is provided.

2. A laser scanning microscope with an AOTF in the laser engagement-beam entry, whereby a heating or a cooling of the AOTF and/or its ambient neighborhood is carried out.

3. A laser scanning microscope in accord with claim 2, wherein the heating or cooling is maintained at a constant, controlled value.

4. A laser scanning microscope in accord with claim 3, wherein the heating brought up to a value above the expected conditions of a corresponding laboratory.

5. A laser scanning microscope in accord with claim 4, whereby the temperature value lies above 35 °C.

6. A laser scanning microscope in accord with one of the claims 2 to 5, whereby in the neighborhood of the AOTF or proximal thereto, or connected to said AOTF, a temperature sensor is provided.

7. A laser scanning microscope in accord with one of the claims 2 to 6, whereby the temperature sensor is connected to an electronic controller for the regulation of the temperature or cooling.

8. A laser scanning microscope in accord with Claim 1, whereby the temperature sensor is connected to a control unit for the AOTF.

Hereto 2 pages of drawings

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